Original Article

The clinical and immunological characteristics related to salivary gland enlargement in primary Sjögren's syndrome: a retrospective cross-sectional study

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Background: Salivary gland enlargement (SGE) is one of the common manifestations in primary Sjögren's syndrome (pSS) patients who are first referred to the hospital of stomatology. Whether the characteristics of the pSS patients with SGE differ from those of the ones without SGE remains unclear. Therefore, this study aimed at investigating the clinical and immunological characteristics related to SGE in pSS, to provide a comprehensive understanding of the clinical phenotype of pSS with SGE.

Methods: In this retrospective cross-sectional study, medical records of patients diagnosed with pSS from 2016 to 2021 were evaluated. The included patients were divided into the SGE and non-SGE groups. Patient data including general clinical data, radiographic and B-ultrasound examination data, and immunological data were extracted. Intergroup differences were analyzed using the chi-square test, Fisher's exact test, and non-parametric tests with SPSS 23.0. Binary logistic regression analysis was further performed to determine the factors related to SGE in pSS.

Results: Two hundred and three patients with pSS were included, including 126 and 77 patients with and without SGE, respectively. Univariate analysis showed that compared to the non-SGE group, the SGE group was younger, had dry eye symptom for a longer duration, and had a higher proportion of patients with severe conditions on salivary gland radiography ($P<0.05$). Regarding immunological indicators, the levels of anti-Ro52, anti-SSA (Ro60), and anti-SSB antibodies; immunoglobulin (Ig)G; IgA; and rheumatoid factor (RF) and erythrocyte sedimentation rate (ESR) were significantly higher in the SGE group ($P<0.05$). Binary logistic regression analysis showed that younger age and high anti-Ro52 levels were independent factors related to SGE in pSS.

Conclusions: SGE is highly consistent with increased immunological indicators, reflecting disease activity. pSS patients with SGE were younger than those without. Special attention should be paid to the changes in the anti-Ro52 level since it is an independent factor related to SGE in pSS.

Keywords: Primary Sjögren's syndrome (pSS); salivary gland enlargement (SGE); clinical characteristics; immunological characteristics

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Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune rheumatic disease characterized by a wide spectrum of glandular and extraglandular features that are associated with the production of various autoantibodies in the blood (1). Its characteristic glandular features mainly include dry eyes and mouth and enlargement of the major salivary glands, while extraglandular features include manifestations of the skin, musculoskeletal system, lung, heart, kidneys, thyroid gland, hematologic system, and nervous system (peripheral and central) involvement (2). Salivary gland enlargement (SGE) is a common manifestation of pSS. It has been reported that 28% of pSS patients show chronic or episodic swelling of the major salivary glands at diagnosis (3). It is mainly caused by the destruction of acinar cells by progressive lymphocyte infiltration (4) and is one of the indicators for predicting disease activity according to the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) (5). Swelling of the salivary glands may commence unilaterally; however, bilateral swelling may develop gradually, resulting in diffused swelling and a smooth surface without tenderness on palpation. In affected patients, saliva secretion is reduced and can lead to the complication of bacterial infection, which could manifest as fever, malaise, and anorexia together with an erythematous, tender gland and secretion of purulent saliva (6,7). This makes it difficult to differentiate the condition from chronic obstructive parotitis and chronic recurrent parotitis and may lead to underdiagnosis of pSS and further treatment delay. Moreover, when salivary gland lobules are destroyed and fused, the salivary glands may have a tumor-like appearance, with one or more nodular mass that is medium or soft in texture and without tenderness (8). Major salivary glands with a tumor-like appearance in pSS are often misdiagnosed as salivary gland tumors, leading to unnecessary surgical treatment.

The onset of pSS is often insidious, and its diagnosis is often delayed for 10 years (9). Due to the wide variety of signs and symptoms of pSS and patients' limited understanding of the disease, pSS patients may visit a diverse range of healthcare practitioners, including dentists, ophthalmologists, and rheumatologists at their initial consultation (2). One- to two-thirds of the patients diagnosed with pSS in our hospital of stomatology had chronic SGE as the main complaint. Whether the clinical manifestations, laboratory test results, and prognosis of such patients differ from those of pSS patients without SGE remains unclear. Therefore, in this study, we reviewed medical data to analyze the characteristics of pSS patients with SGE and determine the clinical and immunological characteristics related to SGE in pSS. We present the following article in accordance with the STROBE reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-22-289/rc) (10).

Highlight box

Key findings

- Salivary gland enlargement (SGE) is highly consistent with increased immunological indicators reflecting disease activity.
- Primary Sjögren's syndrome (pSS) patients with SGE were younger than those without.
- Anti-Ro52 is an independently related factor for SGE in pSS.

What is known and what is new?

- SGE is one of the common manifestations in pSS patients who are first referred to the hospital of stomatology.
- A series of clinical and immunological characteristics in the patients of pSS with SGE clinical subtype are different from those in patients without SGE, such as early age at diagnosis and high anti-Ro52 level.

What is the implication, and what should change now?

- Special attention should be paid to the changes in the anti-Ro52 level since it is an independent factor related to SGE in pSS.

Methods

Study design and population

This study was a retrospective cross-sectional study. The study population was derived from patients who were admitted to the Department of Radiology, Department of Oral and Maxillofacial Surgery, and Department of Oral Medicine in Peking University School and Hospital of Stomatology with a complaint of dry mouth or SGE and suspected of having pSS from January 2016 to

December 2021. Patient data were retrieved retrospectively from medical records. The inclusion criteria were as follows: (I) patients who met the 2016 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for pSS with a score greater than or equal to 4 (11) and (II) patients of either gender aged higher than 18 years. The exclusion criteria were as follows: (I) patients who did not undergo further examinations related to pSS or the examinations were not comprehensive; (II) patients who underwent further examinations related to pSS but did not meet the diagnostic criteria and were diagnosed with other diseases; and (III) patients whose data were incomplete for diagnosis or grouping.

Group assignment

The included pSS patients were divided into SGE and non-SGE groups, which depended on the manifestation of SGE recorded in physical and radiological examinations of major salivary glands.

Ethical clearance

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Peking University School and Hospital of Stomatology (Beijing, China; No. PKUSSIRB-202277081). Individual consent for this retrospective analysis was waived.

Data collection

General clinical data

General condition data and salivary and lacrimal gland involvement data including the patients' age at diagnosis, gender, manifestation of SGE, and duration of dry mouth and eye symptoms were directly extracted from the patients' medical records. Furthermore, extraglandular manifestations of pSS including the involvement of the skin, joints and muscles, thyroid, respiratory system, digestive system, kidney, reproductive system, peripheral and central nervous systems, circulatory system, oral cavity, as well as sleep and mental problems were determined by the diagnosis and symptoms documented in the medical records.

Radiographic and B-ultrasound examination data

The X-ray sialographic stage of pSS was determined according to the criteria established by Rubin and Holt (12,13): stage 0, normal manifestation with or without delayed emptying function; stage 1, diffuse, punctate areas of sialectasis, 1 mm or less in diameter; stage 2, globular sialectasis, 1–2 mm in diameter; stage 3, cavitary sialectasis, more than 2 mm in diameter; and stage 4, destructive pattern. In the present study, the radiographic manifestations were divided into two types according to the sialographic staging method described above, stage 0 was defined as normal or mild type, and the rest were defined as severe type. *Figure 1* shows typical radiographic manifestations of the different types. The parotid and submandibular glands were divided into normal- and abnormal-appearing glands based on B-ultrasound examination manifestations, according to the modified scoring system described by Cornec *et al.* A score higher than 2 was considered to indicate an abnormal appearance (14).

Immunological data

The immunological data of the pSS patients, including the levels of serum autoantibodies, rheumatoid factor (RF), immunoglobulins, and complement component 3 (C3), were extracted. Serum autoantibodies were determined using an immunoblot assay kit (EUROLINE: ANA Profile 3, EUROIMMUN, Lübeck, Germany) that could quantitatively detect 15 autoantibodies including antibodies for nRNP/Sm, Sm, SSA (Ro60), Ro52, SSB, Scl-70, PM-Scl, Jo-1, centromere protein B (CENP-B), proliferating cell nuclear antigen (PCNA), nucleosomes, histones, ribosomal P-proteins, mitochondrion, and intractable cellular antigen dsDNA. The antibody intensity levels were divided into four grades according to the grayscale values of the immunoblot assay, determined using the EUROLineScan software. Specifically, grayscale values from 0 to 10 were defined as negative. Grayscale values greater than 10 were defined as positive: grayscale values from 11 to 50 were scored as 1+ (grade 1), from 51 to 90 as 2+ (grade 2), and those greater than 90 as 3+ (grade 3). RF level was determined using an immunoturbidimetric latex assay (Jiuqiang Biological Co., Ltd. China). Immunoglobulins (Ig)G, M, and A, and C3 levels were determined by immunoscatter turbidimetry (Zhongsheng Beikong Biological Co., Ltd., China).

Bias avoidance

Great effort was made to reduce potential sources of bias. First, to decrease the selection bias, strict inclusion and

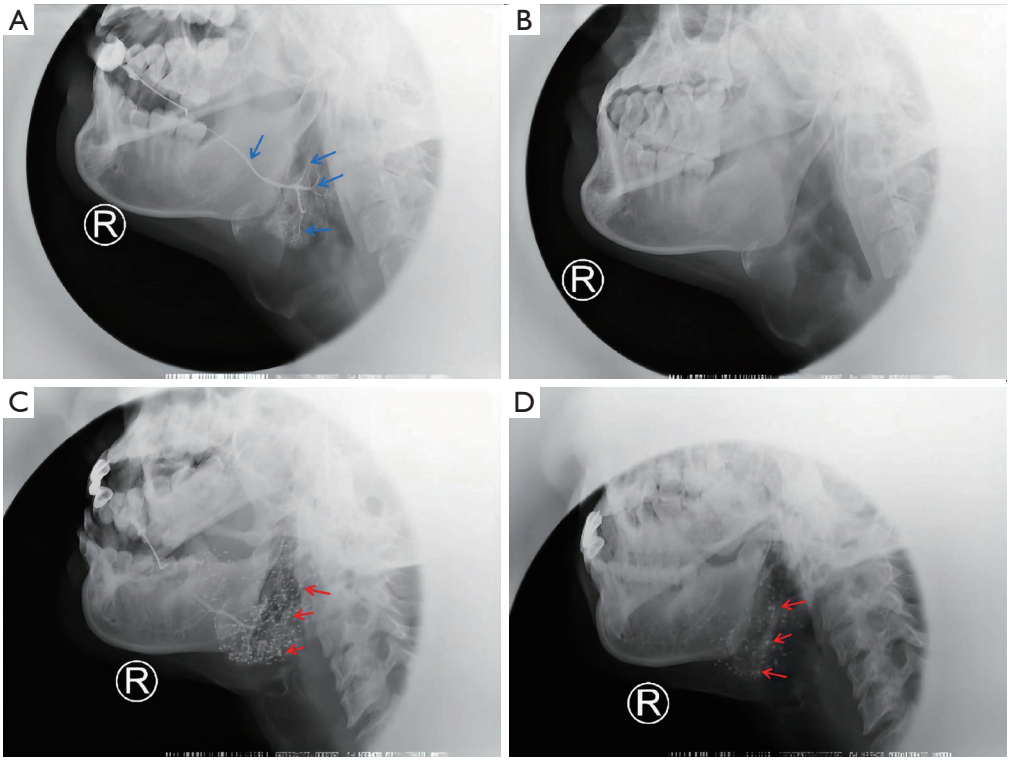

Figure 1 Radiographic manifestations in pSS patients. Mild-type manifestation in a 34-year-old male pSS patient: (A) the contrast phase showed that the main duct and its branches gradually became thinner with a natural course and smooth duct wall, similar to leaf veins. Blue arrows: main duct and its branches. (B) The emptying phase showed substantial emptying of the contrast in the main duct and its branches. Severe-type manifestation in a 48-year-old female pSS patient: (C) the contrast phase showed multiple punctate and globular sialectasis of terminal ducts. Red arrows: multiple punctate and globular sialectasis of terminal ducts. (D) The emptying phase showed delayed emptying with residual contrast in terminal ducts. Red arrows: residual contrast in terminal ducts. pSS, primary Sjögren's syndrome.

exclusion criteria were formulated as previously mentioned. Furthermore, to avoid information bias, the data collection was conducted by two independent investigators. The disagreements were resolved by discussion, and consensus was achieved through the involvement of additional investigators. In addition, multivariate analysis via the binary logistic regression analysis was conducted to decrease the confounding bias.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 23.0 (SPSS 23.0, SPSS Inc., Chicago, IL, USA), and significance was defined as $P<0.05$. Descriptive statistics with frequencies and percentages were used for qualitative variables. Quantitative variables are expressed as [mean (standard deviation)] values if they were normally distributed, and as [median (25% quartile, 75% quartile)] if they were not. For variables with less than 15% of missing variables, the missing data were imputed using means if they were normally distributed and with medians if they were not. Data with more than 15% of missing variables were analyzed using descriptive statistics only. Differences between groups were analyzed using the chi-square or Mann-Whitney rank-sum test, and Fisher's exact test was used when the frequency of categorical variables was less than 5. Factors having a P value <0.05 in the univariate analysis along with clinically relevant confounders were included in the multivariate analysis via binary logistic regression analysis. Both forward and backward conditional methods were used, which served as a sensitivity analysis.

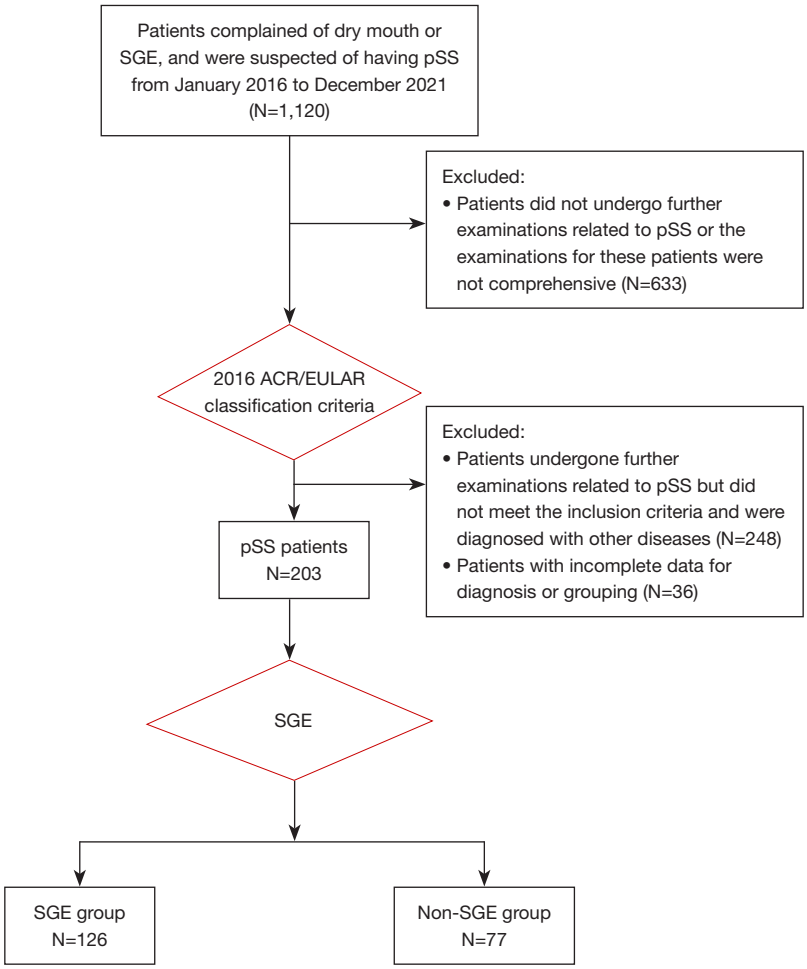

Figure 2 The flow diagram of pSS patient selection. SGE, salivary gland enlargement; pSS, primary Sjögren's syndrome; ACR, American College of Rheumatology; EULAR, European League Against Rheumatism.

Results

Study population

By searching the medical records of patients who were admitted to the Department of Radiology, Department of Oral and Maxillofacial Surgery, and Department of Oral Medicine in Peking University School and Hospital of Stomatology from January 2016 to December 2021, we initially identified 1,120 patients who complained of dry mouth or SGE and were suspected of having pSS. Of these, 633 patients did not undergo further examinations related to pSS or the examinations for these patients were not comprehensive. Two hundred and forty-eight patients underwent further examinations related to pSS but did not meet the inclusion criteria and were diagnosed with other diseases, such as IgG4-related disease, radiation-induced xerostomia, and amyloidosis. The data of 36 patients were incomplete for diagnosis or grouping. Finally, 203 pSS patients who met the 2016 ACR/EULAR classification criteria were included in the study: 126 pSS patients were included in the SGE group and 77 in the non-SGE group (*Figure 2*).

Clinical characteristics

The clinical characteristics of the included pSS patients are presented in *Table 1*. Among the included 203 pSS patients, 12 were male and 191 were female (M:F ratio: 1:15.92), and the mean age was 52 [36, 61] years. All 126 patients in the SGE group developed parotid gland enlargement, of whom 56 and 59 showed bilateral and unilateral enlargement, respectively. There was no record of the type of

Table 1 Comparison of the clinical features of pSS patients with and without SGE

Characteristics	All (n)	SGE	Non-SGE	P value
Gender, n (%)				0.061
Male	12 (5.91)	11 (8.73)	1 (1.30)	
Female	191 (94.09)	115 (91.27)	76 (98.70)	
Age at diagnosis (years) [25%, 75% quartile]	52 [36, 61]	44.5 [31, 55]	61 [51, 66]	<0.001***
Parotid glands enlargement, n (%)				
Bilateral symmetrical enlargement		56 (44.44)		
Unilateral enlargement		59 (46.83)		
Not indicated		11 (8.73)		
Pubmandibular glands enlargement, n (%)				
Bilateral symmetrical enlargement		15 (11.90)		
Unilateral enlargement		0 (0.00)		
Dry mouth symptom duration (m)[b] [25%, 75% quartile]	24 [12, 60]	36 [7.5, 60]	24 [12, 72]	0.163
Dry eyes symptom duration (m)[b] [25%, 75% quartile]	12 [0, 12]	12 [6, 24]	6 [0, 12]	0.001***
LSG biopsy[a], n (%)				
FLS and FS ≥1 foci/4 mm^2	39 (97.50)			
No FLS and FS	1 (2.50)			
UWS[a], n (%)				
UWS ≤0.1 mL/min	86 (94.51)			
UWS >0.1 mL/min	5 (5.49)			
Schirmer's test[a], n (%)				
Schirmer's test ≤5 mm/5 min	53 (91.38)			
Schirmer's test >5 mm/5 min	5 (8.62)			
OSS[a], n (%)				
OSS <5	37 (94.87)			
OSS ≥5	2 (5.13)			
Extraglandular manifestations, n (%)				0.944
Presence	155 (76.35)	96 (76.19)	59 (76.62)	
Absence	48 (23.65)	30 (23.81)	18 (23.38)	
Sleep and mental symptoms, n (%)				0.460
Presence	32 (15.76)	18 (14.29)	14 (18.18)	
Absence	171 (84.24)	108 (85.71)	63 (81.82)	
Family history, n (%)				0.059
Presence	14 (6.90)	12 (9.52)	2 (2.6)	
Absence	189 (93.1)	114 (90.48)	75 (97.4)	

***, P<0.001; [a], LSG biopsy, UWS, Schirmer's test, and OSS were not analyzed between the groups; [b], dry mouth duration [9 (4.43%) missing], dry eyes duration [26 (12.81%) missing]. pSS, primary Sjögren's syndrome; SGE, salivary gland enlargement; LSG, labial salivary gland; FLS and FS, focal lymphocytic sialadenitis and focus score; UWS, unstimulated whole saliva flow rate; OSS, ocular staining score.

enlargement in the case of 11 patients. Submandibular gland involvement occurred in only 15 of the 126 patients; in all of these instances, the involvement was bilateral. Most of the patients (192/203, 94.58%) presented with dry mouth, with the mean symptom duration being 24 [12, 60] months. Dry eye symptom with a mean duration of 12 [0, 12] months was noted in 72.91% of the patients with pSS (148/203). The unstimulated whole saliva flow rate was tested in 91 patients; the rate was less than 1 mL/10 min in 86 patients (94.51%). Labial salivary gland biopsies were conducted in 40 patients, of whom 39 had focal lymphocytic sialadenitis and a focus score (FS) of higher than or equal to 1 foci/4 mm^2 (97.50%). Schirmer's test was performed in 58 of 203 pSS patients, of whom 53 (91.38%) tested positive. Moreover, 39 patients with pSS underwent an ocular staining test, of whom 37 (94.87%) tested positive. Extraglandular manifestations were found in 96 (76.19%) and 59 (76.62%) patients in the SGE and non-SGE groups, respectively. There was no statistical difference in extraglandular involvement between the two groups (P>0.05).

The age at diagnosis in the SGE group was lower than that in the non-SGE group {44.5 [31, 55] *vs.* 61 [51, 66], P<0.001}, and the duration of dry eye symptom in the former was longer {12 [6, 24] *vs.* 6 [0, 12], P=0.001} than that in the latter. There were no differences between the two groups in terms of gender and dry mouth symptom duration (P>0.05).

Radiographic, B-ultrasound, and immunological characteristics

The radiographic, B-ultrasound, and immunological characteristics of the patients are shown in *Table 2*. The proportion of severe-type findings in salivary gland sialography in the SGE group was higher than that in the non-SGE group, and the difference was statistically significant (P<0.05). Twelve patients clinically showed tumor-like changes of parotid glands and were all assigned to the severe type of the SGE group by X-ray sialography (12/126, 9.52%), while two of them were finally diagnosed with pSS complicated by lymphoma. Among the 75 pSS patients who underwent salivary gland B-ultrasound examination, all patients from the SGE group had glands with an abnormal appearance. Regarding the non-SGE group, 30 patients had glands with an abnormal appearance while one patient had normal-appearing glands.

Among the 203 pSS patients, 83 patients (40.89%) showed positive results for all the three autoantibodies including anti-SSA (Ro60), anti-SSB, and anti-Ro52 antibodies, and 83 patients (40.89%) showed positive results for both anti-SSA (Ro60) and anti-Ro52 antibodies. Meanwhile, 11 patients (5.42%) only showed positive results for anti-SSA (Ro60), 9 (4.43%) only showed positive results for anti-Ro52, and one (0.49%) only showed positive results for anti-SSB. The highest autoantibody-positive rate (179/203, 88.18%) was found for anti-SSA (Ro60). The second- and third-highest autoantibody-positive rates were found for anti-Ro52 (177/203, 87.19%) and anti-SSB (84/203, 41.38%). Meanwhile, the anti-SSB positive rate in the SGE group (62/126, 49.21%) was higher than that in the non-SGE group (22/77, 28.57%). Conversely, the anti-CENP-B positive rate in the SGE group (15/126, 11.90%) was lower than that in the non-SGE group (20/77, 25.97%).

The anti-SSA (Ro60) level in the SGE group was higher than that in the non-SGE group (P<0.05), and the proportion of grades 2 and 3 anti-SSA (Ro60) in the SGE group was higher than that in the non-SGE group (105/126, 83.33% *vs.* 40/77, 51.95%; P<0.05). Moreover, the Ro52 grade 3 proportion in the SGE group (106/126, 84.13%) was higher than that in the non-SGE group (38/77, 49.35%).

As for immunoglobulins, the IgG and IgA levels in the SGE group were higher than those in the non-SGE group [IgG: 21.45 (18.57, 24.85) *vs.* 17.54 (14.18, 22.11), P<0.001; IgA: 3.03 (2.49, 3.52) *vs.* 2.87 (2.15, 3.32), P=0.023], and the IgM and C3 levels in the two groups showed no significant differences (P>0.05).

The RF level in the SGE group was also higher than that in the non-SGE group [37.97 (27.29, 104.61) *vs.* 24.38 (4.24, 35.97), P<0.001]. Moreover, the erythrocyte sedimentation rate (ESR) value in the SGE group was higher than that in the non-SGE group {20 [15, 31] *vs.* 20 [10, 25], P<0.05}.

Multivariate analysis of factors involved in pSS

Multivariate analysis via binary logistic regression was conducted to test the effect of each covariant. Age and anti-Ro52 level were identified as independent variables associated with SGE in pSS (*Table 3*). The odds ratio (OR) values of the age calculated by the two models were both 0.947, and the 95% confidence intervals (CIs) were narrow and below 1 (95% CI: 0.924–0.971 and 0.924–0.970, respectively). The OR values of anti-Ro52 grade 1 were 6.432 (95% CI: 1.245–33.237) and 5.519 (95% CI:

Table 2 Comparison of radiographic and immunological features of pSS patients with and without SGE

Characteristics	All (n)	SGE	Non-SGE	P value
Conventional X-ray sialography, n (%)				0.024*
Mild type	7 (3.45)	1 (0.79)	6 (7.79)	
Severe type	196 (96.55)	125 (99.21)	71 (92.21)	
SGUS[a], n (%)				
Normal appearing	1 (1.33)	0 (0.00)	1 (3.23)	
Abnormal appearing	74 (98.67)	44 (100.00)	30 (96.77)	
AMA-M2, n (%)				0.296
Negative	191 (94.09)	121 (96.03)	70 (90.91)	
Grade 1	8 (3.94)	3 (2.38)	5 (6.49)	
Grade 2	4 (1.97)	2 (1.59)	2 (2.60)	
Anti-ribosomal P-proteins, n (%)				0.902
Negative	196 (96.55)	121 (96.03)	75 (97.40)	
Grade 1	7 (3.45)	5 (3.97)	2 (2.60)	
Anti-histone antibodies, n (%)				0.527
Negative	201 (99.01)	124 (98.41)	77 (100.00)	
Grade 1	2 (0.99)	2 (1.59)	0 (0.00)	
Anti-nucleosome antibodies, n (%)				0.774
Negative	198 (97.54)	123 (97.62)	75 (97.40)	
Grade 1	4 (1.97)	2 (1.59)	2 (2.60)	
Grade 2	1 (0.49)	1 (0.79)	0 (0.00)	
Anti-dsDNA antibodies, n (%)				0.952
Negative	194 (95.57)	121 (96.03)	73 (94.81)	
Grade 1	9 (4.43)	5 (3.97)	4 (5.19)	
Anti-PCNA antibodies, n (%)				0.379
Negative	202 (99.51)	126 (100.00)	76 (98.70)	
Grade 1	1 (0.49)	0 (0.00)	1 (1.30)	
Anti-CENP-B antibodies, n (%)				0.037*
Negative	168 (82.76)	111 (88.10)	57 (74.03)	
Grade 1	3 (1.48)	1 (0.79)	2 (2.60)	
Grade 2	2 (0.99)	1 (0.79)	1 (1.30)	
Grade 3	30 (14.78)	13 (10.32)	17 (22.08)	
Anti-Jo-1 antibodies, n (%)				1
Negative	199 (98.03)	123 (97.62)	76 (98.7)	
Grade 1	3 (1.48)	2 (1.59)	1 (1.30)	
Grade 2	1 (0.49)	1 (0.79)	0 (0.00)	

Table 2 (*continued*)

Table 2 (*continued*)

Characteristics	All (n)	SGE	Non-SGE	P value
Anti-PM-Scl antibodies, n (%)				1
Negative	202 (99.51)	125 (99.21)	77 (100.00)	
Grade 1	1 (0.49)	1 (0.79)	0 (0.00)	
Anti-Scl-70 antibodies, n (%)				0.527
Negative	201 (99.01)	124 (98.41)	77 (100.00)	
Grade 1	2 (0.99)	2 (1.59)	0 (0.00)	
Anti-SSB antibodies, n (%)				0.014*
Negative	119 (58.62)	64 (50.79)	55 (71.43)	
Grade 1	50 (24.63)	37 (29.37)	13 (16.88)	
Grade 2	28 (13.79)	19 (15.08)	9 (11.69)	
Grade 3	6 (2.96)	6 (4.76)	0 (0.00)	
Anti-Ro52 antibodies, n (%)				<0.001***
Negative	26 (12.81)	3 (2.38)	23 (29.87)	
Grade 1	15 (7.39)	8 (6.35)	7 (9.09)	
Grade 2	18 (8.87)	9 (7.14)	9 (11.69)	
Grade 3	144 (70.94)	106 (84.13)	38 (49.35)	
Anti-SSA (Ro60) antibodies, n (%)				<0.001***
Negative	24 (11.82)	5 (3.97)	19 (24.68)	
Grade 1	34 (16.75)	16 (12.70)	18 (23.38)	
Grade 2	120 (59.11)	86 (68.25)	34 (44.16)	
Grade 3	25 (12.32)	19 (15.08)	6 (7.79)	
Anti-Sm antibodies, n (%)				1
Negative	198 (97.54)	123 (97.62)	75 (97.40)	
Grade 1	5 (2.46)	3 (2.38)	2 (2.60)	
Anti-RNP/Sm antibodies, n (%)				0.445
Negative	185 (91.13)	113 (89.68)	72 (93.51)	
Grade 1	9 (4.43)	5 (3.97)	4 (5.19)	
Grade 2	7 (3.45)	6 (4.76)	1 (1.30)	
Grade 3	2 (0.99)	2 (1.59)	0 (0.00)	
ESR[b] (25%, 75% quartile)	20 [13, 29]	20 [15, 31]	20 [10, 25]	0.017*
IgG[b] (25%, 75% quartile)	20.34 (16.42, 23.99)	21.45 (18.57, 24.85)	17.54 (14.18, 22.11)	<0.001***
IgA[b] (25%, 75% quartile)	3.03 (2.28, 3.49)	3.03 (2.49, 3.52)	2.87 (2.15, 3.32)	0.023*
IgM[b] (25%, 75% quartile)	1.29 (0.93, 1.76)	1.29 (0.98, 1.77)	1.29 (0.81, 1.65)	0.504

Table 2 (*continued*)

Table 2 (*continued*)

Characteristics	All (n)	SGE	Non-SGE	P value
C3[b] (25%, 75% quartile)	1.32 (1.20, 1.47)	1.32 (1.19, 1.48)	1.32 (1.19, 1.48)	0.786
RF[b] (25%, 75% quartile)	35.97 (15.27, 73.90)	37.97 (27.29, 104.61)	24.38 (4.24, 35.97)	<0.001***

[a], SGUS was not analyzed between the groups; [b], ESR [24 (11.82%) missing], IgG [19 (9.36%) missing], IgA [27 (13.30%) missing), IgM [27 (13.30%) missing], C3 [19 (9.36%) missing], RF [23 (11.33%) missing]; *, P<0.05; ***, P<0.001. Grade 1: grayscale values from 11 to 50 and scored as 1+; Grade 2: grayscale values from 51 to 90 and scored as 2+; Grade 3: grayscale values greater than 90 and scored as 3+. pSS, primary Sjögren's syndrome; SGE, salivary gland enlargement; SGUS, salivary gland ultrasonography; AMA-M2, anti-mitochondrial M2; PCNA, proliferating cell nuclear antigen; CENP-B, centromere protein B; ESR, erythrocyte sedimentation rate; Ig, immunoglobulins; C3, complement component 3; RF, rheumatoid factor.

Table 3 Multivariate analysis by binary logistic regression

Factors	P value	OR	95% CI	
			Lower confidence limit	Upper confidence limit
Forward conditional method				
Age	0.000***	0.947	0.924	0.971
Anti-Ro52				
Negative	0.001***			
Grade 1	0.026*	6.432	1.245	33.237
Grade 2	0.065	4.519	0.913	22.359
Grade 3	0.000***	12.566	3.411	46.296
Backward conditional method				
Age	0.000***	0.947	0.924	0.970
Anti-Ro52				
Negative	0.002*			
Grade 1	0.044*	5.519	1.044	29.185
Grade 2	0.056	4.831	0.958	24.362
Grade 3	0.000***	11.643	3.136	43.230

*, P<0.05; ***, P<0.001. OR, odds ratio; CI, confidence interval.

1.044–29.185), respectively. Moreover, the OR values of anti-Ro52 grade 2 were 4.519 (95% CI: 0.913–22.359) and 4.831 (95% CI: 0.958–24.362), respectively. The OR values of anti-Ro52 grade 3 were 12.566 (95% CI: 3.411–46.296) and 11.643 (95% CI: 3.136–43.230), respectively, and were significantly higher. The Hosmer–Lemeshow statistic was used to assess model goodness-of-fit, and the P value range for the two models was 0.212 and 0.301, indicating acceptable goodness-of-fit.

Discussion

SGE was previously considered to be observed in approximately one-third of patients with pSS (3). However, the proportion of pSS patients with SGE in the present study (62.07%) was significantly higher. A previous study confirmed that 79% of SS patients have salivary gland damage (15). Meanwhile, SGE, which reflects salivary gland destruction, belong to the category of oral manifestation

of pSS, and pSS patients with SGE are often first referred to the hospital of stomatology. Therefore, we speculated that the high involvement of salivary glands in pSS and the affected patients' tendency to visit a dental hospital may be the reasons for the high proportion of pSS patients with SGE in the present study. Moreover, we found that pSS patients with SGE were younger than those without {44.5 [31, 55] *vs.* 61 [51, 66]; P<0.001}. Similarly, Goules *et al.* found a higher frequency of SGE among young pSS patients than among middle-aged pSS controls. Furthermore, the young pSS patient group was characterized by B cell hyperactivity and more robust B cell responses (16). Since SGE in pSS is mainly caused by the destruction of acinar cells due to progressive lymphocyte infiltration (4), the characteristic B lymphocyte hyperactivity in young patients may be its cause.

SS seriously affects the patients' quality of life and often leads to sleep disorders and mental illnesses. The pSS patients included in this study had a high proportion of patients with anxiety, depression, and sleep disorders (32/203, 15.76%). Mental disorders are currently considered to be part of the disease process of pSS, rather than a response to its unpleasant symptoms. The secretion of pro-inflammatory cytokines such as interferon-γ is increased in patients with pSS, and these pro-inflammatory factors may contribute to the development of anxiety and depression. Moreover, nocturnal autonomic symptoms such as palpitations and sweating, which are common in patients with pSS, may affect sleep and lead to sleep disorders (17,18).

This study investigated the positive rates of 15 serum autoantibodies in pSS patients and found that the positive rate of anti-SSA (Ro60), as a diagnostic indicator, was the highest (88.18%), and the anti-SSA (Ro60) level of the SGE group was higher than that in the non-SGE group. The positive rate of anti-SSA (Ro60) in the present study was higher than the positive rates reported in the previous literature, which ranged from 56.9% to 74.8% (19,20). These variations may be related to the differences in diagnosis criteria and the origin of the patients across different studies. Anti-Ro52 was the second most common autoantibody in pSS patients (177/203, 87.19%). In the past, both Ro52 and Ro60 were considered to belong to SSA. More recently, the two different target proteins have been differentiated since they have distinct biochemical and immunological functions, and are associated with different clinical phenotypes (21,22). Robbins *et al.* showed that the positive rate of anti-Ro52 (75.7%) was higher than that of anti-SSA (Ro60) (56.9%) in the autoimmune disease population, and anti-Ro52 was frequently positive in pSS patients (20). In the present study, the positive rate of anti-Ro52 was slightly lower than that of anti-SSA (Ro60) (87.19% *vs.* 88.18%), and 9 patients (4.43%) and 11 patients (5.42%) showed positive results for anti-Ro52 or anti-SSA (Ro60) alone, indicating that the two antibodies may function differently. Moreover, Murng *et al.* found positive results for anti-Ro52 without anti-Ro60 in patients with significant clinical conditions including malignancies, despite the absence of any indications of autoimmunity at the time of testing (23). Consistently, in the present study, anti-Ro52 positive status alone with high levels was found in two pSS patients who were eventually diagnosed with lymphoma. Therefore, a high anti-Ro52 level may play an early predictive role in the development of lymphoma in pSS patients with SGE. Due to the inclusion of a limited number of pSS patients with lymphoma, the predictive role of anti-Ro52 remains to be verified in future large-sample, prospective studies.

In this study, the positive rate of anti-SSB was 41.38%, and the median level of pSS patients with SGE was higher than that of the patients without SGE. We analyzed the positive status of anti-SSA (Ro60), anti-SSB, and anti-Ro52 in pSS patients, and found that only one pSS patient was positive for anti-SSB alone, while the remaining 83 patients showed positive results for anti-SSB in combination with anti-SSA (Ro60). Notably, none of the anti-SSB positive patients were positive for anti-Ro52, which may indicate that the functions of anti-Ro52 and anti-SSB may not be closely related in the development of pSS. Interestingly, we also found that pSS patients with SGE had a lower positive rate for anti-CENP-B. CENP-B is an important structural and functional element for accurate chromosome segregation during cell mitosis (24). The reason remains unclear and will be an important aspect of our further research. Moreover, the concentrations of IgG, IgA, and RF, and the ESR of the SGE group were higher than those without. Since an increase in these indicators reflects the patient's enhanced B cell activation with antibody production (25), SGE may be a predictor of increased disease activity in pSS.

In addition, we also analyzed the correlation between autoantibodies and SGE in pSS by multivariate analysis with binary logistic regression, and found that positive anti-Ro52, not anti-SSA or anti-SSB, was an independent factor related to SGE in pSS, with a correlation coefficient (OR value) of up to 12.566 in the forward conditional method. Our findings are consistent with those found in a

previous animal study, which demonstrated that the epitope specificity of anti-Ro52 plays a critical role in the induction of glandular dysfunction (26). As for the mechanism, the autoantibody induced by Ro52 deposition in the salivary gland may play an important role in the induction of destruction of the salivary gland in pSS patients (27). Another study also found that anti-Ro52-positive pSS patients experience a more severe disease condition, and that anti-Ro52 is an independent risk factor for interstitial lung disease in pSS (28). The glandular domain is an ESSDAI scoring item (5), and the highest score for SGE is 2 points, whereas the anti-Ro52 level is not included in the disease activity evaluation index. Our study demonstrated that the anti-Ro52 level and SGE are promising indicators for assessing pSS activity, so we propose to add anti-Ro52 level as a scoring indicator and increase the weight of the SGE in the disease activity assessing guidelines.

In this study, we included pSS patients who were first referred to the university hospital center of stomatology and comprehensively analyzed the clinical and immunological characteristics. Moreover, the included pSS patients underwent serum immunological examinations, which were performed in the same laboratory with the same methods. Hence, the clinical and immunological results obtained in the study are highly representative of the findings in the Chinese population. Nevertheless, this study has some limitations. Its retrospective nature may have led to selection bias, and the relatively low integrity of other indicators such as the data of the ophthalmological examination, labial gland biopsy, and salivary flow rate of the patients could lead to information bias. Furthermore, the sample size of the study may be too small to draw firm conclusions. Therefore, multicenter, prospective studies with large sample sizes are needed to verify the results of this study. Finally, this study did not address the treatment and prognosis of pSS, and therefore, future research should focus on these aspects because they may have guiding significance for pSS treatment.

Conclusions

SGE is highly consistent with increased immunological indicators reflecting disease activity. Thus, SGE can be used as a clinical indicator reflecting the disease activity of pSS. A series of clinical and immunological characteristics in the patients of pSS with SGE clinical subtype are different from those in patients without SGE, such as early age at diagnosis and high anti-Ro52 level. Since anti-Ro52 is an independently related factor for SGE in pSS, special attention should be paid to changes in the anti-Ro52 level.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://gs.amegroups.com/article/view/10.21037/gs-22-289/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Peking University School and Hospital of Stomatology (Beijing, China; No. PKUSSIRB-202277081). Individual consent for this retrospective analysis was waived.

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